Research Journal of Agricultural Sciences
An International Journal

Res. Jr. of Agril. Sci. **12**(2): 517–520

P- ISSN: 0976-1675 | **E**- ISSN: 2249-4538

www.rjas.org

Research Paper

Effect of Leaves Ethanol Extract of *Elytraria acaulis* Lindau. on Antioxidants and Carbohdyrate Metabolizing Enzymes of Streptozotocin Induced Albino Wistar Rats: *In-vivo* Approach

N. Kiruthika*[1] and M. Shabana Begum[2]

Received: 17 Jan 2021 | Revised accepted: 15 Mar 2021 | Published online: 23 Mar 2021

A B S T R A C T

Plants are the excellent source of various herbal medicines useful in dealing with a variety of human disorders. All-natural products from plants are conventionally used for the treating of diabetes mellitus mostly in developing countries where resources are restricted and usage of modern-day treatment method is a problem. Contra-diabetic activity of the medicinal plants is scientifically examined by in vitro and then in vivo studies. The present examine evaluates the anti-diabetic activity of *Elytraria acaulis* Lindau which are part of the family Acanthaceae. *Elytraria acaulis* Lindau is usually active in dealing with temperature, venereal conditions and cause can be used in mammary tumor, pneumonia, abscesses and infantile diarrhea. The purpose of this study is to elucidate the role leaves of *Elytraria acaulis* on glucose impairing metabolism during diabetes by *in-vivo* methods. Diabetes was stimulated in adult rats of the Wistar strain by intraperitoneal injection of streptozotocin (STZ) (45 mg/kg) and the trial rats were treated with leaves ethanol extract of *Elytraria acaulis* (100, 200 and 300 mg/kg bw) and glibenclamide (10 mg/kg bw) in STZ induced hyperglycemia. On completion of the 60-day treatment, a range of carbohydrate metabolizing enzymes (glucokinase, glucose-6-phosphatase and fructose 1, 6 bisphosphatase) and antioxidants like SOD, GPx, CAT, GSH and LPO were tested including liver of control and STZ diabetic rats. Leaves ethanol extract of *Elytraria acaulis* dose-dependently altered the activities of glucose-6 phosphatase, fructose 1, 6-bisphosphatase and liver glucokinase to very close to control groups as well in antioxidants activities through the oral administration of extract on 300 mg/kg bw. Leaves ethanol extract of *Elytraria acaulis* at doses of 300 mg/kg/day for 60 days provided a major decrease in diabetic difficulties and metabolic impairment.

***Key words*:** *Elytrariaacaulis*, Anti-diabetic, Streptozotocin, SOD, Catalase

Diabetes is a metabolic syndrome causing from a deficiency in insulin secretion, insulin action, or both. Its global occurrence was about 8% in 2011 and is predicted to rise to 10% by 2030 [1]. As metabolic issue is described as a mixture of risk factors for cardiovascular diseases and diabetes that usually link to insulin deficit or dysfunction. Hence, it is essential to find out the role of newly develop antidiabetic principle(s) on that point of view [2].

Elytraria acaulis is one such plant that is frequently being used, the leaves decoction of this plant is prescribed in fever, venereal diseases and root is used in mammary tumor, abscesses, pneumonia, and infantile diarrhea as well as traditional medicine for long days [3]. Leaves used for treating wounds infected with worms. Locally in Tamil Nadu (Tirunelvelli District) it is used as antidiabetic. *Elytraria acaulis* is among the family Acanthaceae is actually a tiny shrub, which expands in shady dry areas. The entire bush is commonly used for medicinal reasons [4]. Despite of the popular use and considerable phytopharmacological reports, the toxicity information, especially on its chronic use, has not been yet explored. However, there are actually no technological studies with regards to the negative effects of this mangrove vegetation on Diabetes mellitus. In impact, we aimed to create mangrove flower therapy as antidiabetic drug instead of chemical drug. Therefore, from the present examine, we assessed the impact of your results in ethanol extract of *Elytraria acaulis* Lindau. (*Ea*L-Et) in streptozotocin-induced diabetes rats for that potential use in the long-term treatments for diabetes.

*N. Kiruthika
kiruthi405@gmail.com

[1-2]Department of Biochemistry, Muthayammal College of Arts and Science, Rasipuram - 637 408, Namakkal District, Tamil Nadu, India

MATERIALS AND METHODS

Collection and authentication of the plant material

Fresh, mature, *Elytraria acaulis* were collected from Namagiripettai. The plant was further identified and authenticated by a taxonomist, ABS Herbal Garden, Vidya nagar, Salem-3.

Preparation of extraction

The coarse powder of the plant material was extracted and soaked with ethanol. The solvent was removed under reduced pressure to get the crude extract.

Assay for in vivo hypoglycemic activity

Animals

Experiments were done on adult male Wistar rats (body weight range: 150-180 g). Animals were housed and preserved at 22°C within a 12:12 light/darker cycle, with cost-free access to water and food. Experiments have been conducted during the standard light/dark period and also started at the exact same hour. Attempts were designed to decrease animal enduring and also to reduce the volume of animals employed. All tests complied with the Ethical Guidelines for the Use of Animals in Research, the review was licensed by the community Internal Committee to the Care and Use of Laboratory Animals of Muthayammal School of arts of arts and Science, Rasipuram, India. (1416/PO/a/11/CPCSEA&7 MARCH 2011)

Induction of diabetes and experimental design

Streptozotocin at dose level of 45mg/kgbw was applied to a group of overnight adult Albino Wistar rats, tends to make pancreas enlarge and also at triggers weakening in Langerhans islet beta tissues and induces experimental diabetes mellitus in the 48-72 hours [5]. Following 72 hours, the induction of diabetes was verified by evaluating the glucose excreted in the urine of streptozotocin induced rats.

Study design

In order to determine the hypoglycemic effect of *E. acaulis* leaves in diabetic rats, oral doses of *Ea*L-Et (100, 200 and 300 mg/kg) were administered via oral for study period. The rats found with permanent diabetes were used for the antidiabetic study. Animals have been divided into six groups of six rats each. The extract was administered for 60 days. Feed and drinking water were actually presented *ad libitum* towards the animals. The plant extract (*Ea*L-Et) or glibenclamide had been dissolved in drinking water and post orally utilizing an intra gastric pipe on the treatment of 60 days.

Group I: Normal control rats treated with saline daily for 60 days.
Group II: Diabetic control rats induced by STZ (45 mg/kg bw).
Group III: Diabetic rats treated with (100 mg/kg) of *Ea*L-Et.
Group IV: Diabetic rats treated with (200 mg/kg) of *Ea*L-Et.
Group V: Diabetic rats treated with (300 mg/kg) of *Ea*L-Et.
Group VI: Diabetic rats treated with glibenclamide (10 mg/kg)

Assay for carbohydrate metabolic enzymes

After 60 days of treatment, the rats were actually anesthetized and reduced by cervical decapitation. The liver was dissected out and cleaned with ice-cold saline immediately to remove blood. The Glucokinase (EC2.7.1.1) [6], Glucose-6-phosphatase (EC 3.1.3.9) [7] and Fructose-1, 6-bisphosphatase [8] (EC3.1.3.11) might be the essential enzymes in blood sugar levels homeostasis which had been predicted to look for the ultimate outcome of *Ea*L-Et and handling of diabetes. For such distinctive assessments, 1 g of new/frosty liver was sliced and homogenized in an ice-cold sucrose (15 ml, 250 mM) simply by using a Potter-Elvehjem homogenizer for 2 minutes, centrifuged at 10 000 rev./min for eo minutes, combined with the pellet was thrown away as well as the supernatant was used because the source for the biochemical estimation of enzymes.

Determination of antioxidant levels

In all group animals, Liver collected after scarification and washed immediately with an ice-cold saline to take out blood vessels. The antioxidants, superoxide dismutase (SOD) [9], glutathione peroxidase (GPx) [10], catalase (CAT) [11], reduced glutathione (GSH) [12] and lipid peroxidation by creating thiobarbituric acid reactive substances (TBARS) [13] had been determined in liver.

Statistical analysis

All data are expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) was carried out combined with Tukey's assessment to compare the versions between treatments. Differences are believed to be statistically significant for $p<0.05$.

RESULTS AND DISCUSSION

Medicinal plant affords a broad area for restorative application from the beginning, but many in the medicinal pursuits yet being investigated. Estimation of physical and harmful consequences, solvent used for removal, path of management, and acute or chronic impact of *Elytraria acaulis* leave extract are usually diverse, which can be encouraged by delineating the advantageous applications of the study plant and confines various evaluations.

Effect on carbohydrate metabolic enzymes

Results in the administration of *Ea*L-Et and glibenclamide on glucokinase, glucose-6-phosphatase, and fructose-1, 6-bisphosphatase of liver are provided in (Table 1). The activity of hepatic glucokinase is appreciably lowered while glucose-6-phosphatase and fructose-1, 6-bisphosphatase are significantly increased in streptozotocin-induced diabetic rats as opposed to normal rats [14]. It comes with an improved activity of glucokinase and decreased the pursuits of glucose 6-phosphatase and fructose-1, 6- bisphosphatase within the administration of *Ea*L-Et (100, 200 and 300 mg of bw) and glibenclamide in contrast to diabetes rats.

Table 1 Effect of *Ea*L-Et on glucokinase, glucose 6 phosphatase and fructose 1,6 bisphosphatase of experimental animals

Groups	Glucokinase	Glucose 6 phosphatase	Fructose 1,6 Bisphosphatase
I	3.66 ± 0.28	5.67 ± 0.77	53.41 ± 1.74
II	12.70 ± 0.40*	11.20 ± 0.55*	134.78 ± 1.54*
III	10.84 ± 0.50	9.13 ± 0.44	99.26 ± 1.75
IV	7.26 ± 0.52	7.38 ± 0.62	75.42 ± 2.72
V	3.90 ± 0.55**	5.84 ± 0.42**	55.42 ± 1.75**
VI	3.53 ± 0.47**	5.28 ± 0.37**	54.24 ± 1.52**

Glucokinase = µmole of Glucose liberated /min/mg protein; Glucose 6 phosphatase = µmole of Pi liberated/min/mg protein; Fructose 1, 6Bisphosphatase = µM of Glucose utilized/min/mg protein
Values are Mean ± SE, n = 6
*p<0.05 statistically significant when compared with Group I;
**p<0.05 statistically significant when compared to Group II.

In-vivo antioxidant activity

The antioxidant activity of *Ea*L-Et in Liver was analyzed in diabetic rats along with the data displayed in (Table 2). After the induction of diabetes by STZ,

considerably (P<0.05) decreased levels of SOD, CAT, GPx, reduced GSH and improved level of TBARS in Liver were actually seen compared to normal control rats [15]. These changed above antioxidant levels have been reversed substantially (P<0.05) to near normal levels right after the administration of *Ea*L-Et 300 mg/kg dosage and glibenclamide 10 mg/kg dose when compared with diabetes control rats.

Table 2 Effect of *Ea*L-Et on GSH, SOD and CAT of experimental animals

Groups	SOD	CAT	GPx	GSH	LPO
I	11.3 ± 0.71	47.6 ± 2.5	5.6 ± 0.43	0.71 ± 0.05	0.25 ± 0.01
II	6.25 ± 0.52*	33.6 ± 2.4 a*	3.83 ± 0.32*	0.35 ± 0.02*	0.39 ± 0.01*
III	7.52 ± 0.82	37.6 ± 1.85	4.21 ± 0.52	0.48 ± 0.06	0.33 ± 0.02
IV	9.45 ± 0.74	39.5 ± 2.24	4.95 ± 0.31	0.56 ± 0.04	0.27 ± 0.01**
V	10.5 ± 0.56**	41.4 ± 3.23**	5.24 ± 0.84**	0.61 ± 0.04**	0.24 ± 0.01
VI	11.6 ± 0.7 **	47.8 ± 2.04**	5.7 ± 0.42**	0.69 ± 0.04**	0.23 ± 0.01**

SOD = U/mg protein; CAT = U/mg protein; GPx = U/mg protein; GSH = µg/ g tissue; LPO=nmol MDA/g tissue.
Values are Mean ± SE, n = 6;
*p<0.05 statistically significant when compared with Group I; **p<0.05 statistically significant when compared to Group II.

Streptozotocin (STZ) is often accustomed to induce experimental diabetes in animals [16]. STZ-induced diabetes may be because of vitiate glucose oxidation and lowering of insulin biosynthesis and secretion. The toxicity of STZ is because of DNA alkylation from the methyl nitrosourea moiety mainly at O6 position of guanine [17]. The exchange of methyl group from STZ to the DNA molecule causes injury which results in fragmentation of DNA and efficient defects in the beta cells [18]. Additionally, STZ is potential to act as an intracellular nitric oxide (NO) donor and creates reactive oxygen species (ROS) [19]. The synergistic action of both NO and ROS might also bring about DNA fragmentation along with other deleterious adjustments caused by STZ [20].

The intracellular glucose has become used by insulin in many ways. The increased level of insulin impacts the activity of gluconeogenic enzymes that brings about the initiation of hepatic glycolysis [21]. Glucokinase is a lot more distinct for glucose and differ along with other forms of hexokinase in kinetic and regulatory properties, that has been seen in hepatocytes [22]. Hexokinase performs a core role within the maintenance of glucose homeostasis, it catalyzes the conversion of blood glucose to glucose-6-phosphate [23]. Also, hexokinase is an important regulator of glucose storage and disposal from the liver [24]. In the present study, the hexokinase activity was lowered in streptozotocin-diabetic rats which might be because of insulin deficiency (insulin energizes and activates glucokinase). Treatment with *Ea*L-Et or glibenclamide heightened the activity of more efficient restorative ingredients. Fructose 1, 6 bisphosphatase is among the significant enzymes of gluconeogenic pathway plus it stimulates the level limiting step of fructose 1-6-bisphosphate to fructose-6-phosphate [25]. Hepatic blood sugar generation is increased in diabetes status and is associated to the impaired suppression of your gluconeogenic enzyme fructose 1, 6-bisphosphatase [26]. Annoyed overall performance of carbohydrate metabolising enzymes in diabetic rats denoted that this carbohydrate metabolic pathways (glycolysis, glycogenolysis, glycogenesis and gluconeogenesis) were harshly disturbed this is probably due to insulin deficit in body [27]. Improved level of fructose 1, 6 bisphosphatase in STZ induced diabetic rats was seen and this could be accountable for decreased glycolysis and improved gluconeogenesis which leads to using glucose for energy production [28]. Administration of *Ea*L-Et brought back the enzyme activation indicates its normalizing outcome in diabetes.

A variety of studies revealed that oxidative stress takes on a significant function within the development, progression of diabetes as well as its associated problems [29]. In diabetic express, free radical generation may take place via improved glycolysis, intercellular activation of polyol pathway, auto-oxidation of glucose and non-enzymatic healthy proteins glycation [30]. Furthermore, severe lowering of *in vivo* antioxidant enzymes level in various tissues were documented in diabetes condition [31]. In our study, diminished levels of liver and renal SOD, CAT, GPx, GSH as well as increased level of TBARS had been seen in STZ-induced diabetes rats compared to normal control rats. The reduction of above enzymes directly demonstrates the oxidative stress in diabetic rats and they enzyme level changes might be due to generation of free radicals by polyol pathway, auto-oxidation of glucose, glycosylation in hyperglycemic situation in as well as STZ mediated generation of ROS by its NO donor property to the intracellular molecules [32]. In the present study, increased SOD, CAT, GSH and GPx levels in addition to reduced TBARS level have been discovered in diabetes rats after the administration of both doses of *Ea*L-Et and glibenclamide in liver and renal system. The aforementioned action symbolizes the antioxidant property of *Ea*L-Et in diabetic condition and hence, *Ea*L-Et possesses a possible to lessen or prevent the diabetic micro and macrovascular difficulties.

CONCLUSIONS

In conclusion, this is the first report on *Elytraria acaulis* Lindau on antidiabetic activity in experimental model. Further our study data confirm that *E. acaulis* possesses blood glucose lowering action in diabetic condition. Moreover, it has hypolipidemic and antioxidant activities in diabetic state, therefore it has an ability to prevent diabetic complications. Hence, above findings have given scientific evidence to the traditional use of *Elytraria acaulis* Lindau leaves in the treatment of diabetes.

LITERATURE CITED

1. Varsha M, Smita M, Nomita G, Manisha K. 2018. Diabetes: The next epidemic? *International Journal of Life Sciences* 6(2): 665-680.
2. Martín-Timón I, Sevillano-Collantes C, Segura-Galindo A, del Cañizo-Gómez FJ. 2014. Type 2 diabetes and cardiovascular disease: have all risk factors the same strength? *World Journal of Diabetes* 5(4): 444.

3. Koshy RK, Kapoor BR, Azamthulla M. 2012. Anti-hyperglycemic activity of *Elytraria acaulis* Lind. On Streptozotocin-induced diabetic rats. *Medicinal and Aromatic Plants* 1: 103.
4. Sherman RA, Hall MJR, Thomas S. 2000. Medicinal maggots: an ancient remedy for some contemporary afflictions. *Annual Review of Entomology* 45(1): 55-81.
5. Balamurugan R, Ignacimuthu S. 2011. Antidiabetic and hypolipidemic effect of methanol extract of *Lippia nodiflora* L. in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine* 1(1): 30-36.
6. Brandstrup N Kirk JE, Bruni C. 1959. Determination of hexokinase in serum in liver diseasea *Clin. Chem. Acta* 4: 554-561.
7. Swanson MA. 1955. *Methods in Enzymology*. (Eds) S.P. Colowick and N.O. Kaplan, Academic Press Inc., New York. pp 2: 541.
8. Gancedo JM, Gancedo C. 1971. Fructose 1-6 diphosphate, Phosphofructokinas and Glucose 6 phosphate dehydrogenase. *Soc. Exp. Biol. Med.* 106: 607-609.
9. Misra HP, Fridovich I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry* 247(10): 3170-3175.
10. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra W. 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179(4073): 588-590.
11. Maehly AC, Chance B. 1954. *Methods of Biochemical Analysis.* (Eds) Glick D. New York, *Interscience*. pp 357.
12. Moron MS, Depierre JW, Mannervik B. 1979. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. *Biochim. and Biophys. Acta* 5820: 60-68.
13. Ohakawa H Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95: 351-358.
14. Gurudeeban S, Satyavani K, Ramanathan T, Balasubramanian T. 2012. Antidiabetic effect of a black mangrove species *Aegiceras corniculatum* in alloxan-induced diabetic rats. *Journal of Advanced Pharmaceutical Technology and Research* 3(1): 52.
15. Reddy SS, Ramatholisamma P, Karuna R, Saralakumari D. 2009. Preventive effect of *Tinospora cordifolia* against high-fructose diet-induced insulin resistance and oxidative stress in male Wistar rats. *Food and Chemical Toxicology* 47(9): 2224-2229.
16. Zari TA, Al-Attar AM. 2007. Effects of ginger and clove oils on some physiological parameters in streptozotocin-diabetic and non-diabetic rats. *Journal of Medical Sciences* 7(2): 265-267.
17. Ramachandran S, Rajasekaran A, Manisenthilkumar KT. 2012. Investigation of hypoglycemic, hypolipidemic and antioxidant activities of aqueous extract of *Terminalia paniculata* bark in diabetic rats. *Asian Pacific Journal of Tropical Biomedicine* 2(4): 262-268.
18. Vanitha P, Senthilkumar S, Dornadula S, Anandhakumar S, Rajaguru P, Ramkumar KM. 2017. Morin activates the Nrf2-ARE pathway and reduces oxidative stress-induced DNA damage in pancreatic beta cells. *European Journal of Pharmacology* 801: 9-18.
19. Hsieh HJ, Liu CA, Huang B, Tseng AH, Wang DL. 2014. Shear-induced endothelial mechanotransduction: the interplay between reactive oxygen species (ROS) and nitric oxide (NO) and the pathophysiological implications. *Journal of Biomedical Science* 21(1): 1-15.
20. Friedman M. 2014. Chemistry and multi-beneficial bioactivities of carvacrol (4-isopropyl-2-methylphenol), a component of essential oils produced by aromatic plants and spices. *Journal of Agricultural and Food Chemistry* 62(31): 7652-7670.
21. Han HS, Kang G, Kim JS, Choi BH, Koo SH. 2016. Regulation of glucose metabolism from a liver-centric perspective. *Experimental and Molecular Medicine* 48(3): 218-e218.
22. Iynedjian PB. 2009. Molecular physiology of mammalian glucokinase. *Cellular and Molecular Life Sciences* 66(1): 27-42.
23. Srinivasan S, Sathish G, Jayanthi M, Muthukumaran J, Muruganathan U, Ramachandran V. 2014. Ameliorating effect of eugenol on hyperglycemia by attenuating the key enzymes of glucose metabolism in streptozotocin-induced diabetic rats. *Molecular and Cellular Biochemistry* 385(1): 159-168.
24. Khan MW, Priyadarshini M, Cordoba-Chacon J, Becker TC, Layden BT. 2019. Hepatic hexokinase domain containing 1 (HKDC1) improves whole body glucose tolerance and insulin sensitivity in pregnant mice. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1865(3): 678-687.
25. Yánez AJ, Nualart F, Droppelmann C, Bertinat R, Brito M, Concha II, Slebe JC. 2003. Broad expression of fructose-1, 6-bisphosphatase and phosphoenolpyruvate carboxykinase provide evidence for gluconeogenesis in human tissues other than liver and kidney. *Journal of Cellular Physiology* 197(2): 189-197.
26. Pari L, Satheesh MA. 2006. Effect of pterostilbene on hepatic key enzymes of glucose metabolism in streptozotocin-and nicotinamide-induced diabetic rats. *Life Sciences* 79(7): 641-645.
27. Pari L, Murugan P. 2005. Effect of tetrahydrocurcumin on blood glucose, plasma insulin and hepatic key enzymes in streptozotocin induced diabetic rats. *Journal of Basic and Clinical Physiology and Pharmacology* 16(4): 257-274.
28. Wu C, Khan SA, Peng LJ, Lange AJ. 2006. Roles for fructose-2, 6-bisphosphate in the control of fuel metabolism: beyond its allosteric effects on glycolytic and gluconeogenic enzymes. *Advances in Enzyme Regulation* 46(1): 72-88.
29. Asmat U, Abad K, Ismail K. 2016. Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharmaceutical Journal* 24(5): 547-553.
30. Jaganjac M, Tirosh O, Cohen G, Sasson S, Zarkovic N. 2013. Reactive aldehydes–second messengers of free radicals in diabetes mellitus. *Free Radical Research* 47(Sup1): 39-48.
31. Amalan V, Vijayakumar N, Indumathi D, Ramakrishnan A. 2016. Antidiabetic and antihyperlipidemic activity of p-coumaric acid in diabetic rats, role of pancreatic GLUT 2: *In vivo* approach. *Biomedicine and Pharmacotherapy* 84: 230-236.
32. Kowluru RA, Mishra M. 2015. Oxidative stress, mitochondrial damage and diabetic retinopathy. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1852(11): 2474-2483.